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**Exocrine glands in the legs of the social wasp *Vespula vulgaris***

Catherine Nijs & Johan Billen

*Zoological Institute, University of Leuven, Naamsestraat 59, box 2466, B-3000  
Leuven (Belgium)*

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**Corresponding author:**

Johan Billen, University of Leuven, Zoological Institute, Naamsestraat 59, box 2466,  
B-3000 Leuven, Belgium  
Tel: (32) 16 323975  
Fax: (32) 16 324575  
E-mail: Johan.Billen@bio.kuleuven.be

## Abstract

This study brings a survey of the exocrine glands in the legs of *Vespula vulgaris* wasps. We studied workers, males, virgin queens as well as mated queens. A variety of 17 glands is found in the different leg segments. Among these, five glands are novel exocrine structures for social insects (trochanter-femur gland, ventrodistal tibial gland, distal tibial sac gland, ventral tibial gland, and ventral tarsomere gland). Most leg glands are present in the three leg pairs of all castes. This may indicate a mechanical function. This is likely for the numerous glands that occur near the articulation between the various leg segments, where lubricant production may be expected. Other possible functions include antenna cleaning, acting as a hydraulic system, or pheromonal. Further research including leg-related behavioural observations and chemical analyses may help to clarify the functions of these glandular structures in the legs.

## 1. Introduction

Apart from some specific functions such as predation (e.g. mantis and predatory bugs), digging (e.g. mole crickets) or pollen collection (e.g. bees and bumblebees), the legs form the major means of locomotion that insects use in addition to their wings. Although this may involve special developments such as rowing devices in water beetles or massive femora for jumping in grasshoppers, most insects have slender legs for walking. It is obvious that the legs contain muscle and nerve fibres to control the necessary movements. It may be less evident to find also other tissues inside the legs, although a recent review revealed that ants can harbor up to 20 different exocrine glands in their legs (Billen, 2009). It was already known that also bees can have a dozen glands in their legs (Cruz Landim et al., 1998; Cruz-Landim, 2002), while a follow-up study in workers of just a single stingless bee species *Frieseomelitta varia* revealed 15 different glands in their legs (Billen and Vander Plancken, 2014). The only known reports on the occurrence of glands in wasp legs are the description of the arolium glands in *Polistes annularis* workers and queens (Billen, 1986) and a paper by Beani and Calloni (1991) who found scattered gland cells in the tarsomeres, tibia and femur of males and females of *Polistes dominulus*. This overwhelming variety of glands in the legs of ants and bees inspired us to examine and compare the situation in the legs of wasps as the third major Hymenopteran group of social insects.

## 2. Material and methods

Wasps of the three castes (3 individuals each of workers, virgin and mated queens, and males) were obtained from nests that were collected in Leuven, Belgium. In order to allow proper penetration of the various solutions during tissue preparation, we made transverse cuts of each leg in the femur, tibia and basitarsus. This resulted in smaller tissue pieces formed by coxa-trochanter-femur, femur-tibia, tibia-basitarsus, and the distal tarsomeres. The leg fragments were fixed in 2% glutaraldehyde (buffered at pH 7.3 with 50 mM Na-cacodylate and 150 mM saccharose), followed by postfixation in 2% osmium tetroxide in the same buffer. This was followed by dehydration in a graded acetone series and embedding in araldite.

Serial semithin sections of 1  $\mu\text{m}$  were made with a Leica EM UC6 ultramicrotome using a diamond knife, stained with methylene blue and thionin, and examined with an Olympus BX-51 microscope. Thin sections for electron microscopy with a thickness of 70 nm were double stained with lead citrate and uranyl acetate, and viewed in a Zeiss EM900 microscope. For estimation of cell numbers of class-3 glands, we first calculated the average diameter of the secretory cells for a particular gland by looking at the biggest cells appearing on sections. The cumulative number of cells appearing on serial sections at an interval equivalent to this diameter was then considered as the total number of secretory cells of this particular gland. In addition to the 3 individuals of each caste that were prepared for sectioning, we also mounted the legs of 3 other individuals on stubs, that were gold coated and examined with a JEOL JSM-6360 scanning microscope.

### 3. Results and discussion

We found a total of 17 exocrine glands in the legs of *Vespula vulgaris*, that we present here in the order from most proximal (coxa) to most distal (pretarsus). In our descriptions, we follow the standard classification as given in the pioneer paper of Noirot and Quennedey (1974), that distinguishes between epithelial class-1 glands and class-3 glands, that are formed by a number of bicellular units. Both class-1 and class-3 glands can open through the external tegumental surface, or they can open into a internal reservoir, where secretion can be temporarily stored (Fig. 1). For cell measurements in queens, virgin and mated queens are pooled together, as no clear differences were found between both reproductive conditions. All longitudinal sections are shown with the distal side to the right. A general survey of all glands is given in Figure 2 and Table 1.

#### 3.1. Coxal gland

In their distal part, near the articulation with the trochanter, the coxae of the three leg pairs in all castes contain clusters of spherical class-3 secretory cells (Figs 3A,B). Queens have 50-70 cells with a diameter of  $43.9 \pm 4.2 \mu\text{m}$ , workers have approx. 30 cells with a diameter of  $33.6 \pm 1.0 \mu\text{m}$ , while males have 10-20 cells with a diameter of  $25.1 \pm 4.1 \mu\text{m}$ . The cells have centrally located round nuclei with a

diameter of approx. 10  $\mu\text{m}$ , and occur both at the dorsal and ventral side. Their ducts open through the dorsal and ventral cuticle, as well as through the articulation membrane between coxa and trochanter. The location at this articulation site in the three castes may be indicative for a lubricant function, although the clearly bigger gland in queens, as expressed in both a considerably higher cell size and cell number, may also be linked with a pheromonal function. Ultrastructural examination reveals the occurrence of a well developed smooth endoplasmic reticulum (Fig. 3D), that can be linked with the elaboration of non-proteinaceous lubricant substances.

Similar class-3 secretory cells have also been found in the three leg pairs of *Pachycondyla obscuricornis* ants, where they occur both in the proximal region of the coxa where it articulates with the thorax, as in the distal region near the junction with the trochanter (Schoeters and Billen, 1993). Cruz Landim et al. (1998) described clusters of class-3 secretory cells in the proximal part of the coxae of several meliponine bee species, although the opening site of their ducts could not be traced. Distally occurring coxal glands, with ducts opening through the intersegmental membrane with the trochanter and a presumed lubricant function, as here described for *Vespula vulgaris*, were also reported in *Frieseomelitta varia* stingless bees (Billen and Vander Plancken, 2014). Class-3 coxal glands have also been found in neotenic *Prorhinotermes simplex* termites (Šobotník et al., 2003).

### 3.2. Trochanter-femur gland

The trochanter of the three leg pairs in all castes contains in its distal region a cluster of 10-15 round class-3 secretory cells, their accompanying ducts open through the articulation membrane with the femur (Fig. 3C). They have a diameter of  $35.6 \pm 9.5 \mu\text{m}$  in queens and  $25.6 \pm 4.2 \mu\text{m}$  in workers (no reliable measurements could be made in males), and have spherical nuclei. Their occurrence in the three castes and location near the junction between the trochanter and femur as well as the opening site of the ducts through the articulation membrane possibly indicate a lubricant function.

As no gland cells with this location have been found so far in social insects, we consider this trochanter-femur gland as a novel exocrine structure. The only class-3 cells that have been reported in this region occur in the distal part of the trochanter of meliponine bees, although their opening through the sclerotized trochanter cuticle

(Cruz-Landim, 2002) is different from the intersegmental opening of the novel gland described here.

### 3.3. Leg tendon gland

Along their entire length, the legs display a hollow tendon that functions as a claw retractor. In all legs of all castes, we found a glandular differentiation of the epithelial lining of the tendon in the femur, tibia (Fig. 4C) as well as the basitarsus (Fig. 6A). The tendon lumen acts as reservoir space, and guides secretion to an opening ventrally in the pretarsus. Because of this common opening for its three subparts, we consider the leg tendon gland as a single exocrine structure. Whereas the tendon epithelium elsewhere hardly reaches 1-2  $\mu\text{m}$ , it is considerably thicker in the differentiated glandular regions. In the femur, it has a thickness of  $18.3 \pm 2.4 \mu\text{m}$  in queens,  $17.2 \pm 1.5 \mu\text{m}$  in workers, and  $17.3 \pm 2.6 \mu\text{m}$  in males. In the tibia, the glandular epithelium is  $16.9 \pm 3.2 \mu\text{m}$  in queens,  $15.9 \pm 1.8 \mu\text{m}$  in workers, and  $16.9 \pm 2.7 \mu\text{m}$  in males. In the basitarsus, queens have an epithelial thickness of  $17.7 \pm 3.6 \mu\text{m}$ , workers  $16.4 \pm 0.9 \mu\text{m}$ , and males  $14.3 \pm 0.4 \mu\text{m}$ . Along its entire length, the gland cells of the leg tendon have centrally located round nuclei with a diameter of 5  $\mu\text{m}$ . The glandular differentiation, however, only stretches at one side of the tendon, as can be clearly seen on cross sections (Fig. 4C). The other side retains its thick cuticular lining, thus allowing the tendon to keep its mechanical function as a claw retractor. We observed apical microvilli (Fig. 6B) as well as numerous mitochondria in the glandular epithelium.

Similar leg tendon glands have been described in the femur and tibia of workers of the stingless bee *Melipona seminigra*, where they function in food marking (Jarau et al., 2004). The glands were recently also found in the three castes of *Bombus terrestris* (Jarau et al., 2012). Besides the femur and tibia, also the basitarsus displays the glandular epithelium. Although the specific function in these bumblebees is still elusive, the chemical composition of the hydrocarbons and wax esters in the secretion differs between castes (Jarau et al., 2012). Leg tendon glands in ants have been reported in the tibia of *Crematogaster*, *Amblyopone* and *Formica* species (Billen, 2009). Especially the hindleg ‘tibial gland’ and its function as source of the trail pheromone of *Crematogaster* is well known, and was the first gland described in the legs of ants (Leuthold, 1968; Fletcher and Brand, 1968; Pasteels et

al., 1970). Morgan and co-workers (2004) identified (R)-2-dodecanol as the active substance in the trail pheromone of *Crematogaster castanea*. This pheromonal function for the leg tendon glands in ants, as well as in bees (Jarau et al., 2004), make it tempting to suggest a similar role in wasps, although there so far is no such information available at all. It is noteworthy that wasps are the only group among social insects of which it is not yet known whether and how they communicate information about the location of food sources among nestmates (Raveret Richter, 2000).

### 3.4. Dorsodistal femoral gland

All legs in the three castes contain an accumulation of numerous class-3 cells in the dorsodistal region of the femur, that also extend more proximally (Fig. 3F). Queens are the most conspicuous with 140-160 cells of  $48.3 \pm 6.4 \mu\text{m}$  diameter. Workers and males have 60-80 cells with a diameter of  $32.8 \pm 5.3 \mu\text{m}$  (workers) resp.  $30.4 \pm 2.1 \mu\text{m}$  (males). The gland is especially conspicuous in the hindlegs, with more than 200 cells in queens and approx. 150 in workers and males. The glandular cells contain round to ovoid centrally located nuclei of 8-10  $\mu\text{m}$ , strands of granular endoplasmic reticulum and a branched end apparatus (Fig. 3E). Their accompanying duct cells open through the external dorsodistal cuticle of the femur as small pores of 1  $\mu\text{m}$  diameter (Fig. 3G).

Class-3 gland cells in the distal femoral part have also been found in the three leg pairs of both males and females of *Polistes dominulus*, where they may be involved in scent marking through leg rubbing (Beani and Calloni, 1991). A similar dorsodistal femoral gland was recently also reported in workers of the stingless bee *Frieseomelitta varia*, although it is much less conspicuous with approx. 10 cells of 25  $\mu\text{m}$  (Billen and Vander Plancken, 2014) than we found in *Vespula vulgaris*. Ants may also contain a few class-3 cells, although these are a different exocrine structure as they open through the articulation membrane with the tibia, which may be in line with a lubricant function (Billen, 2009). The conspicuous size, especially in the hindlegs, and the opening at the outer femoral surface of the dorsodistal gland in *Vespula vulgaris* probably are related with a so far unknown behavioral function.

### 3.5. Distal femoral sac gland

In the three leg pairs of all castes, the tegumental epidermis in the ventrodistal part of the femur folds back to form a sac-like gland (Fig. 3F). The epithelium is considerably thicker than elsewhere in the femur, and measures  $26.3 \pm 4.6 \mu\text{m}$  in queens,  $20.0 \pm 2.1 \mu\text{m}$  in workers and  $17.0 \pm 0.9 \mu\text{m}$  in males. The round nuclei have a diameter of 7-8  $\mu\text{m}$  and have a basal to central position in the cells. A cuticular basal plate separates the sac in an upper and a lower part (Fig. 3F). The invaginated epithelium resembles the distal femoral sac gland that has been described in *Harpegnathos saltator* and *Odontoponera transversa* ants (Billen, 2009). We did not observe a clear internal reservoir sac as was found in these ants, however, although the appearance of the epithelium suggests we are dealing with the same exocrine structure in wasps and ants. The gland resembles the distal tibial sac gland (see 3.9) and the arolium gland (see 3.17).

A sacculiform femoral gland has also been reported in some stingless bees, where it only occurs in the queens (Cruz-Landim et al., 1998). This gland, however, occurs proximally in the femur, and therefore is not considered equivalent to the distal femoral sac gland of ants and wasps.

### 3.6. Proximal tibial gland

The epidermis of the dorsoproximal region of the tibia in the three leg pairs of all castes is differentiated into a glandular epithelium (Fig. 4A). It has a thickness of  $19.7 \pm 3.4 \mu\text{m}$  in queens,  $19.7 \pm 2.8 \mu\text{m}$  in workers and  $19.8 \pm 1.5 \mu\text{m}$  in males. The cells have centrally located round nuclei with a diameter of 5  $\mu\text{m}$ . At the ultrastructural level, we find abundant electron-clear spherical vesicles, apical microvilli and cuticular canals (Fig. 4B). As the epithelium is situated near the dorsodistal margin of the femur, and is equally well-developed in all castes, it may play a lubricant role.

A similar proximal tibial gland with presumed lubricant function also occurs in some ponerine ant species (Billen, 2009), but has not been found in other social insects.



### 3.7. Dorsodistal tibial gland

The distal region of the tibia in the three leg pairs of all castes contains clusters of spherical class-3 cells with central round nuclei (Fig. 4D). The appearance of the cells looks similar to that of the dorsodistal femoral gland, with gland cells also occurring more proximally. Queens have approximately 100 cells with a diameter of  $26.8 \pm 2.1 \mu\text{m}$ . Workers and males have approximately 30 cells of  $26.6 \pm 3.6 \mu\text{m}$  resp.  $21.0 \pm 2.7 \mu\text{m}$ . The corresponding duct cells open as small pores through the dorsodistal tibial cuticle. Ultrastructural observation shows a branched end apparatus and isolated strands of granular endoplasmic reticulum (not shown).

Isolated class-3 glands in the distal portion of the tibia were also found in the legs of *Polistes dominulus* males and females, and possibly have a function in communication (Beani and Calloni, 1991). Similar class-3 cells that open through the distal tibial cuticle in ants have been found in bees (Cruz-Landim et al., 1998; Billen and Vander Plancken, 2014) and in *Dorylus* army ants (Billen, 2009). Other ants may contain class-3 cells in the distal tibial region that open through the articulation membrane between tibia and basitarsus, and therefore probably represent a different exocrine structure (Hölldobler et al. (1996) for *Pachycondyla tarsata*, Billen (1997) for *Dorylus molestus*, Tijskens et al. (2002) for *Amblyopone reclinata*).

Bacchus (1979) described clusters of class-3 gland cells that open into sunken pore plates on the distal portion of the tibia of the 6 legs in several Rhinotermitidae termites. The function of these glands is unclear (Bacchus, 1979), although their peculiar opening through distinct pore plates (Costa-Leonardo, 1994; Soares and Costa-Leonardo, 2002) is very different from the dorsodistal tibial glands here reported for wasps and ants.

### 3.8. Ventrodistal tibial gland

We also found in all leg pairs ventrally located class-3 cells with central round nuclei in the distal part of the tibia, of which some open through the articulation membrane with the basitarsus (Fig. 4D) while others open through the ventral tibial tegument. As such ventrally occurring gland cells have not been found before, we consider this a novel gland, that we call the ventrodistal tibial gland. The gland occurs in the three castes, with approx. 40 cells of  $36.8 \pm 4.7 \mu\text{m}$  in queens, 10 cells of  $24.1 \pm 1.1 \mu\text{m}$  in workers and 5 cells of  $22.2 \pm 2.4 \mu\text{m}$  in males. As is also the case with

the dorsodistal tibial gland, the gland is most developed in queens with both more and larger cells, which may be related with an as yet unknown reproductive function. The opening site through both the tegumental cuticle and the articulation membrane with the basitarsus may indicate these are two different exocrine structures. The cells that open through the intersegmental membrane may possible have a lubricant function.

### **3.9. Distal tibial sac gland**

Besides the two class-3 glands, the distal part of the tibia of the three leg pairs in all castes also contains a novel epithelial gland, that is formed by a ventral invagination of the tibial tegumental epidermis (Fig. 4D). It looks similar to the distal femoral sac gland (see 3.5), and is equally separated into an upper and a lower part by a cuticular basal plate. The epithelium has a thickness of  $30.3 \pm 8.8 \mu\text{m}$  in queens,  $22.2 \pm 0.9 \mu\text{m}$  in workers and  $23.8 \pm 5.7 \mu\text{m}$  in males. The rounded nuclei are centrally located and have a diameter around  $10 \mu\text{m}$ . As in its femoral equivalent, we did not notice a clear internal reservoir sac as in ants (Billen, 2009). The resemblance of the femoral and this novel tibial sac gland to the arolium gland (see 3.17) may indicate these glands all perform comparable functions.

### **3.10. Ventral tibial gland**

Only in the forelegs of males, we found another so far unknown ventral epithelial gland (Fig. 4D). It has a thickness of  $17.7 \pm 0.1 \mu\text{m}$ , with centrally located round nuclei with a diameter of  $6\text{--}7 \mu\text{m}$ . A similar gland has not been found before in other social insects. As it only occurs in the male forelegs, it may have an as yet unknown reproductive function in this caste.

### **3.11. Tibial spur gland**

In the forelegs only, the tibial spur of all three castes contains an epithelial gland, that can almost fill the entire spur (Fig. 4E,F). It has a thickness of  $33.7 \pm 13.4 \mu\text{m}$  in queens,  $22.0 \pm 14.1 \mu\text{m}$  in workers and  $44.6 \pm 6.8 \mu\text{m}$  in males. The apical cell membrane shows a clear microvillar border. Cell nuclei are rounded with a diameter of  $7\text{--}8 \mu\text{m}$  and are basally located.

The function of this gland remains unknown, although it is suggestive to think of an eventual involvement in antenna cleaning, possibly together with the antenna cleaner gland (see 3.13). Tibial spur glands have so far only been described in some ant species (Hölldobler et al. (1992) for workers of *Prionopelta amabilis*; Tijskens et al. (2002) for workers of *Amblyopone reclinata*; Billen (2009a) for queens of *Odontoponera transversa*). These were also found in the forelegs, except for *Amblyopone reclinata*, that has a tibial spur gland in its hindlegs (Tijskens et al., 2002).

### 3.12. Proximal tarsomere glands

Queens, workers and males show a glandular differentiation of the proximal dorsal epithelium of tarsomeres 1 to 4 in the three leg pairs (Fig. 5D, 6A). It has a thickness of  $17.6 \pm 2.4 \mu\text{m}$  in queens,  $16.2 \pm 1.5 \mu\text{m}$  in workers and  $12.8 \pm 0.6 \mu\text{m}$  in males. The round nuclei have a diameter around  $5 \mu\text{m}$  and are centrally located. The proximal tarsomere gland is situated underneath the distal margin of the tibia, with which it articulates (Fig. 6A). The gland has a similar position near an articulation as the proximal tibial gland (see 3.6), which may indicate it has a lubricant function.

Similar proximal tarsomere glands are also known in tarsomeres 1 to 4 of ants (both dorsally and ventrally in workers of *Dorylus molestus* (Billen, 1997); only dorsally in queens of *Atta sexdens rubropilosa*, workers of *Dinoponera quadricaps* and both female castes of *Odontoponera transversa* (Billen, 2009)). In several stingless bee species, the entire epidermis of the basitarsus (= t1) shows a glandular differentiation, although its function remains unknown (Cruz Landim and de Moraes, 1994).

### 3.13. Antenna cleaner gland

As in other social Hymenoptera, the forelegs of the three castes of *Vespula vulgaris* wasps are characterized by an antenna cleaner apparatus, that is formed by a comb on the tibial spur and a brush area on the opposite proximal region of the basitarsus (Fig. 5A). We also found a similar structure in the hindlegs of the three castes (Fig. 5B), which has been considered a wing folding device (Edwards, 1980). Underneath the basitarsal brush, a conspicuous epithelium occurs, that is especially prominent in the forelegs, where it has a thickness of  $56.0 \pm 17.6 \mu\text{m}$  in queens,  $71.6$

± 15.3 µm in workers and 40.7 ± 5.7 µm in males. In the hinglegs, the epithelium exists as well, but is considerably thinner (20.2 ± 1.1 µm in queens, 41.1 ± 4.6 µm in workers, 23.8 ± 3.4 µm in males). The epithelium appears as a mixture of columnar glandular cells and sensory cells that are associated with the brush hairs (Fig. 5C foreleg, Fig. 5D hindleg).

The antenna cleaner gland was first described by Janet (1894) in *Myrmica laevinodis* as an externally visible porous band. A detailed study in *Messor rufitarsis* (Schönitzer et al., 1996) revealed the presence of a very conspicuous glandular epithelium, that was called the antenna cleaner gland. Direct involvement in antenna cleaning, however, has not been experimentally proven so far. It has been shown in *Pachycondyla apicalis* that the foreleg basitarsal brush plays a role in the accumulation of hydrocarbons, that are used when the ant cleans itself (Hefetz et al., 2001). Our finding of a similar structure in the hindlegs indicates we are dealing with a homologous structure, for which we use the published name of 'antenna cleaner gland', although its function here definitely has nothing to do with the antennae.

### 3.14. Dorsal tarsomere glands

Tarsomeres 1, 2, 3 and 4 of all legs in the three castes are characterized by an abundance of round class-3 with central nuclei (Figs 5E in t1, 6A in t2-t3, 5J in t3). Their ducts open through the dorsal cuticle as round pores of 1 µm diameter (Fig. 5F), although tarsomeres 1 and 2 also contain similar cells opening through the ventral cuticle (see 3.15). The cells contain a branched end apparatus and a well-developed Golgi apparatus, while other cell organelles are less apparent (Fig. 5G). In queens, the cells have a diameter of 36.7 ± 3.2 µm, and are more numerous in the proximal tarsomeres (for the hindlegs approximately 100 in t1, 80 in t2 and 30 in t3 and t4; the forelegs and midlegs have more or less half of these cell numbers). Workers and males have approximately 30 cells with a diameter of 29.8 ± 1.6 µm and 24.0 ± 2.4 µm, respectively. We also found approx. 5 dorsally occurring gland cells in the proximal part of the pretarsus (t5) of queens, but not in workers or males.

Tarsomere glands with class-3 cells were also found in the various tarsomeres of both males and female *Polistes dominulus* wasps, without information on whether they occurred dorsally or ventrally, but also more pronounced in the hindlegs (Beani and Calloni, 1991). These authors describe how especially males of these wasps

drag their hind tarsi over the substrate possibly for territorial marking. A pheromonal role seems also plausible in *Vespula vulgaris*, as an eventual lubricant function is not very likely considering their opening on the external surface, away from the articulation areas between tarsomeres. The presence and abundance of gland cells in all legs of all castes calls for more close behavioural observations of specific leg movements.

Dorsally located class-3 glands in the various tarsomeres have also been observed in ants (Billen, 2009). Stingless bees, on the other hand, do not contain any class-3 glands in their tarsomeres (Cruz Landim et al., 1998; Billen and Vander Plancken, 2014).

### **3.15. Ventral tarsomere glands**

Besides these dorsal tarsomere glands (3.14), we also found similar but less numerous class-3 gland cells opening through the ventral surface of tarsomeres t1 and t2 (Fig. 5D). Queens have approximately 40 cells ( $30.5 \pm 1.5 \mu\text{m}$ ), workers have 10 cells ( $24.7 \pm 5.4 \mu\text{m}$ ) and males have around 30 cells ( $27.2 \pm 3.2 \mu\text{m}$ ). As for their dorsally occurring counterpart, also these ventral tarsomere glands possibly have a pheromonal function.

We are not aware of any ventrally occurring glands of this kind in other social Hymenoptera, and therefore consider these ventral tarsomere glands a novel exocrine structure. Bacchus (1979) and Costa Leonardo (1994) described sunken pore plates on the ventral surface of tarsomeres t1 and t2 in Rhinotermitidae resp. Serritermitidae termites, that correspond with duct openings of tarsomere glands, although this clustered arrangement is very different of the one we found in wasps. Later, Soares and Costa-Leonardo (2002) and Costa-Leonardo et al. (2015) found a more widespread occurrence of ventral pores on tarsomeres t1-t2-t3 in several termite groups. According to Bacchus (1979), these glands may have a role in defence, whereas Costa-Leonardo et al. (2015) suggest a role in antenna cleaning or production of antibiotics.

### **3.16. Distal tarsomere glands**

The distalmost tegumental epithelium in tarsomeres t1-t4 in all legs of all castes shows a conspicuous thickening (Fig. 5E, 6A,C). In queens, it has a thickness

of  $37.5 \pm 9.0 \mu\text{m}$ ,  $28.1 \pm 3.0 \mu\text{m}$  in workers and  $26.3 \pm 4.4 \mu\text{m}$  in males. The cells have rounded nuclei that occur in the basal part of the cells. As it is located at the junction between tarsomeres, a lubricant function is most likely.

A similar gland has also been described in the various legs of several ant species (Billen, 2009) and in the stingless bee *Frieseomelitta varia* (Billen and Vander Plancken, 2014), where it has also been considered to produce lubricant substances.

### 3.17. Arolium gland

The arolium (= pretarsal) gland is formed by a large sac-like epithelial invagination in the pretarsus (t5) of all legs of all castes. The lumen does not have a connection to the exterior, but extends into the arolium (Fig. 6D). The epithelial lining is made up by cylindrical cells with basally located round nuclei. The cells have a thickness of  $71.9 \pm 9.8 \mu\text{m}$  in queens,  $44.0 \pm 2.2 \mu\text{m}$  in workers and  $37.3 \pm 4.4 \mu\text{m}$  in males.

This is the most common of all leg glands, as we found it in all Hymenopteran species we examined. It was first described by Arnhart (1923) in the honeybee, and was reported to produce an adhesive secretion that was released to the outside through a ventral groove. The existence of such groove was also reported by Chauvin (1962) and Lensky et al. (1985) for honeybees, Cruz Landim and Staurengo (1965) for stingless bees, and Pouvreau (1991) for bumblebees. It was first listed for wasps in *Polistes annularis* by Billen (1986), who questioned the groove connection to the outside. Also ants have arolium glands (Hölldobler and Palmer, 1989a,b; Billen, 2009), but without outside connection. Apart from a role in adhesion, various functions have been attributed to the arolium gland, such as production of a wax-like secretion (Chauvin, 1962) and inhibition of royal cell formation by queen honeybees (Lensky and Slabezki, 1981), and marking of the nest entrance or food sources by bumblebees (Goulson et al., 2000). Detailed studies by Jarau et al. (2004, 2005) in *Melipona seminigra* finally and convincingly illustrated the lack of a connection to the exterior, and showed how the presumed marking function had to be attributed to the leg tendon glands instead. A function in adhesion, as was originally postulated, is probably still the most plausible, although not through presumed adhesive secretions, but through a hydraulic mechanism in which arolium gland liquids are pumped into

the arolium. As a result, the unfolding arolium acts as an adhesive pad by increasing the contact area with the surface (Federle et al., 2001).

## 4. Conclusion

Although literature data on exocrine glands in the legs of wasps so far only mentioned about the existence of some class-3 cells in the femur, tibia and tarsomeres (Beani and Calloni, 1991) and of the arolium gland (Billen, 1986), our present study of *Vespula vulgaris* highlights a much higher diversity of leg glands in wasps. Although most of the glands here reported are new for wasps, five of them represent novel exocrine structures for social insects as such. A peculiar characteristic for the class-3 glands is the presence of a branched end apparatus in the secretory cells. This is a typical feature for class-3 glands in wasps, that is not found in other social insect groups (Van der Vecht's gland: Delfino et al., 1979, Marino Piccioli et al., 1986; venom gland: Delfino et al., 1983, Petrocelli et al., 2014; Richards' gland: da Silva et al., 2014). The precise three-dimensional organization of the end apparatus is often complex, and may be interpreted as a coiled appearance (Křížková et al., 2014), although the branched nature in wasps is supported by electron microscopy observations.

Most glands are found in the three leg pairs of queens, workers and males. Queens usually have the most developed glands, as expressed in both cell size (epithelial thickness for class-1 glands, cell diameter for class-3 glands) and cell number (class-3 glands). This probably is in line with their larger size (queen-worker dimorphism in *Vespula vulgaris* is 1.38: Wenseleers and Ratnieks, 2006), although for some glands the difference is very pronounced, and may therefore reflect a difference function in relation to the queens' reproductive role (coxal gland, dorsodistal femoral gland, dorsodistal and ventrodiscal tibial gland, dorsal tarsomere glands). The common occurrence of most glands in the three castes may be linked with mechanical functions such as the production of lubricant substances. This assumption finds additional support by the frequent occurrence of glands near the articulation between leg segments, where smearing should occur. Several glands that are found near such articulations moreover occur in a repetitive pattern, which further supports a lubricant function. Glands that open through the external surface in

non-articulation regions may have pheromonal functions, as has been suggested for some femoral, tibial and tarsomere glands (Beani and Calloni, 1991). A clear pheromonal function has been shown for the leg tendon glands in ants (Leuthold, 1968), bees (Jarau et al., 2004) and bumblebees (Jarau et al., 2012). Behavioural observations of peculiar leg movements as well as chemical studies of gland secretions may hopefully shed more light on the function of the wide variety of leg glands in social wasps.

## **5. Acknowledgements**

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Table 1. Survey of the various leg glands present in the forelegs (F), midlegs (M) and hindlegs (H) of the three castes of *Vespula vulgaris*, with indication of the gland type following figure 1. As the leg tendon gland is considered as one major gland, its femoral, tibial and basitarsal subparts are all indicated with the same number. Bold lettering indicates novel gland structures, that were not reported in social insects previously. (-) indicates gland absence.

leg part	Nr	gland	worker	queen	male	type
coxa	1	coxal gland	F M H	F M H	F M H	C,E
trochanter	<b>2</b>	<b>trochanter-femur gland</b>	<b>F M H</b>	<b>F M H</b>	<b>F M H</b>	<b>C</b>
femur	3	(femoral part of) leg tendon gland	F M H	F M H	F M H	B
	4	dorsodistal femoral gland	F M H	F M H	F M H	C
	5	distal femoral sac gland	F M H	F M H	F M H	B
tibia	6	proximal tibial gland	F M H	F M H	F M H	A
	3'	(tibial part of) leg tendon gland	F M H	F M H	F M H	B
	7	dorsodistal tibial gland	F M H	F M H	F M H	C
	<b>8</b>	<b>ventrodistal tibial gland</b>	<b>F M H</b>	<b>F M H</b>	<b>F M H</b>	<b>C,E</b>
	<b>9</b>	<b>distal tibial sac gland</b>	<b>F M H</b>	<b>F M H</b>	<b>F M H</b>	<b>B</b>
	<b>10</b>	<b>ventral tibial gland</b>	- - -	- - -	<b>F</b> - -	<b>A</b>
tarsomeres	11	tibial spur gland	F - -	F - -	F - -	A
	12	proximal tarsomere glands	F M H	F M H	F M H	A
	3''	(basitarsal part of) leg tendon gland	F M H	F M H	F M H	B
	13	antenna cleaner gland	F - H	F - H	F - H	A
	14	dorsal tarsomere glands	F M H	F M H	F M H	C
	<b>15</b>	<b>ventral tarsomere glands</b>	<b>F M H</b>	<b>F M H</b>	<b>F M H</b>	<b>C</b>
	16	distal tarsomere glands	F M H	F M H	F M H	A
	17	arolium gland	F M H	F M H	F M H	B

## Figure legends

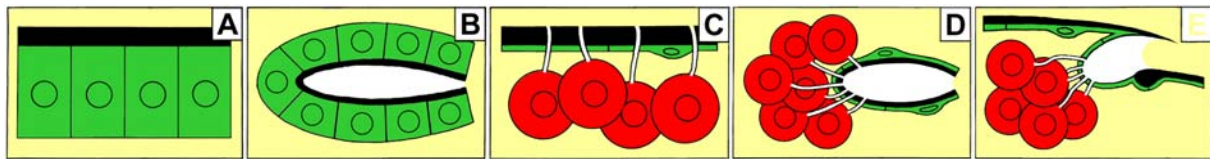


Fig. 1. Schematic view of the main anatomical types of insect glands: (A) Epithelial glands without reservoir. (B) Epithelial glands with reservoir. (C) Bicellular unit glands without reservoir. (D) Bicellular unit glands with reservoir. (E) Bicellular gland units opening through articulation membrane that acts as reservoir space (figure from Billen, 2009).

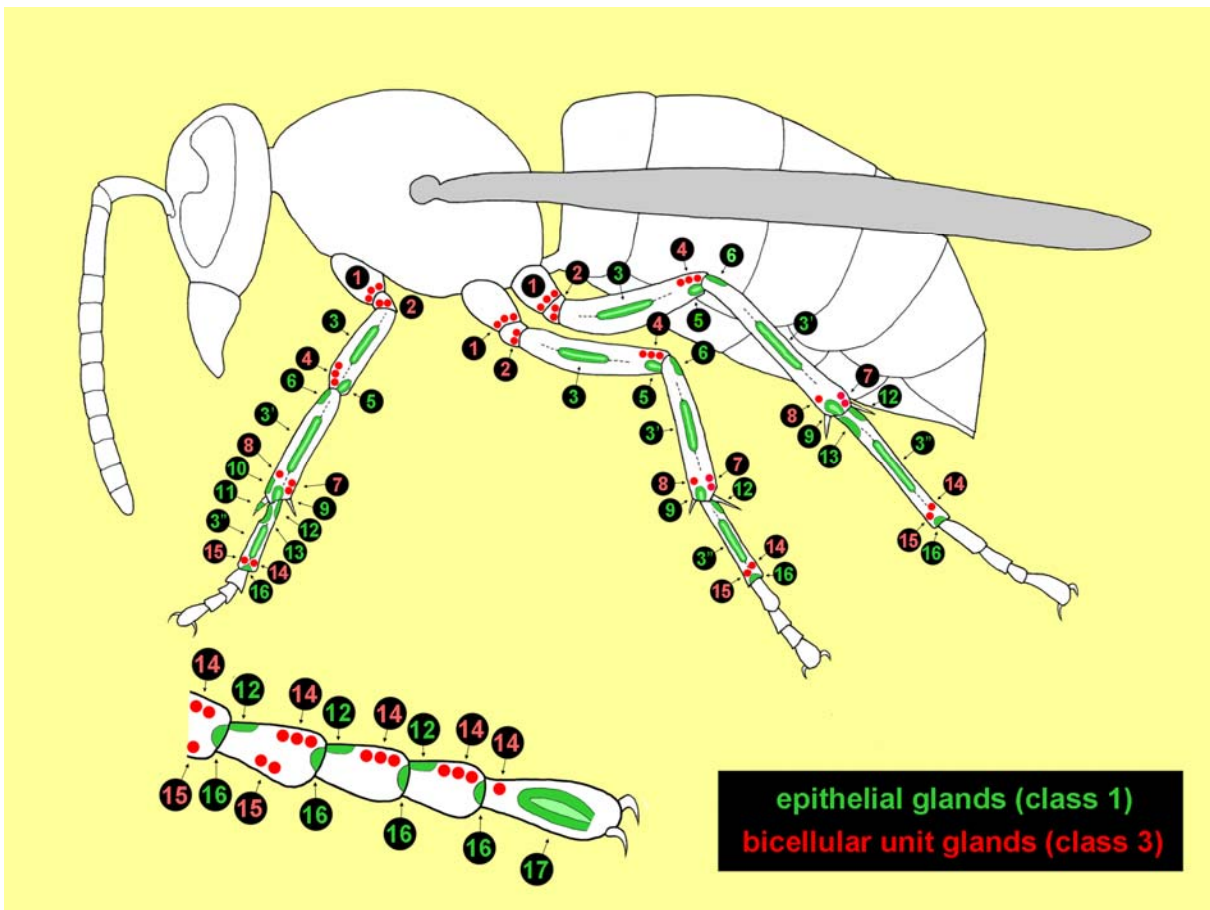
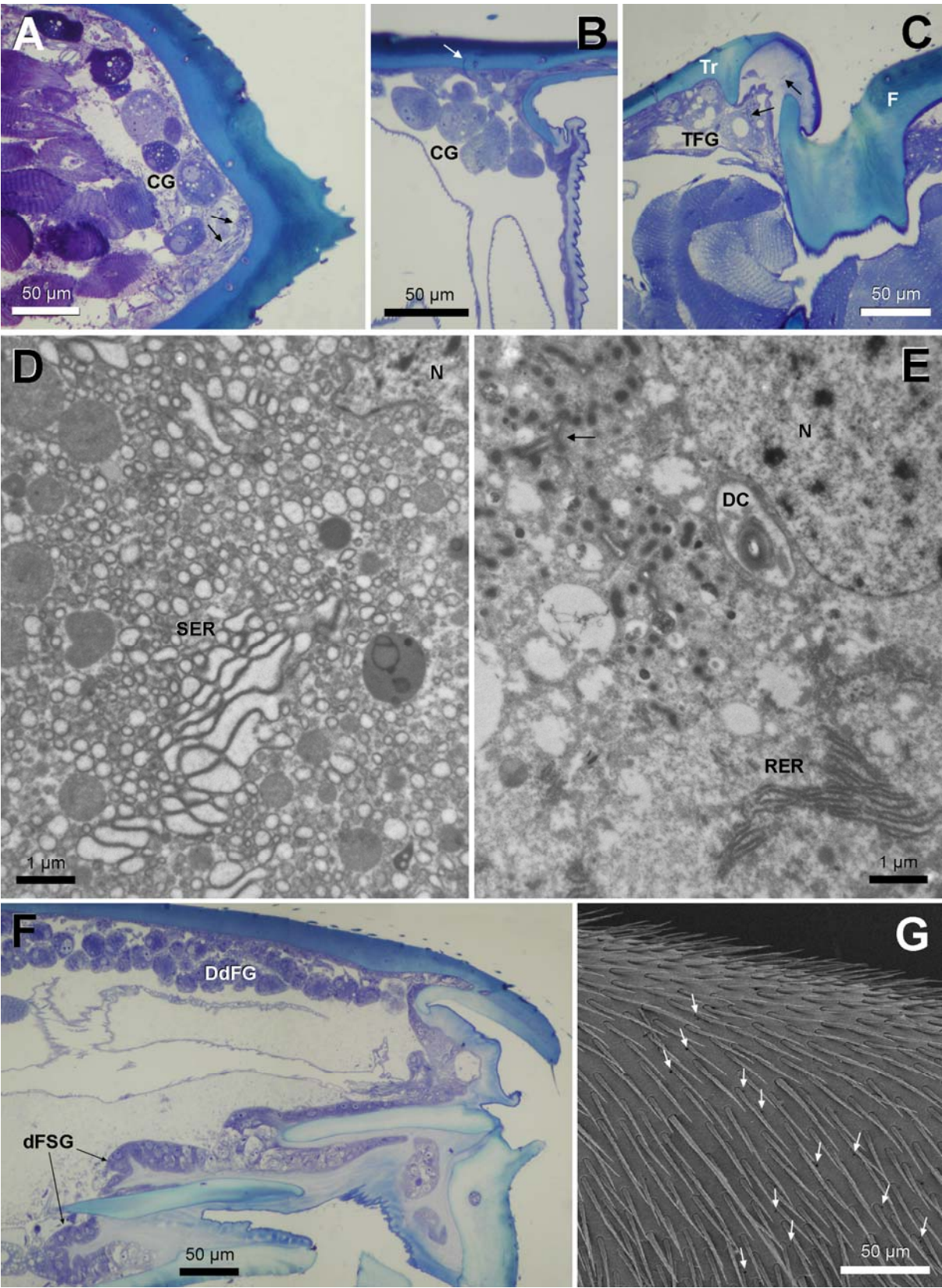


Fig. 2. Schematic profile drawing of *Vespula vulgaris* with indication of the various leg glands. The tarsomere glands are indicated in the enlarged detail drawing. The numbering of the glands corresponds with that used throughout the paper and in Table 1.





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Fig. 3. Glands in coxa, trochanter and femur. **A.** Hind coxa of mated queen with coxal gland cells (CG, arrows show ducts). **B.** Fore coxa of worker with cluster of

602 coxal gland cells (CG, arrow indicates duct). **C.** Junction of trochanter and  
603 femur in worker hindleg, showing trochanter-femur gland (TFG, arrows indicate  
604 ducts). **D.** Ultrastructure of coxal gland cell in mid coxa of mated queen,  
605 showing well-developed smooth endoplasmic reticulum (SER). **E.** Ultrastructure  
606 of dorsodistal femoral gland cell in midleg of virgin queen, arrow indicates  
607 branched end apparatus. **F.** Junction of femur and tibia in worker hindleg,  
608 showing dorsodistal femoral gland (DdFG) and distal femoral sac gland  
609 (DFSG). **G.** Scanning micrograph of dorsodistal part of femur in worker midleg,  
610 showing duct openings of dorsodistal femoral gland (arrows). F: femur, N:  
611 nucleus, Tr: trochanter.



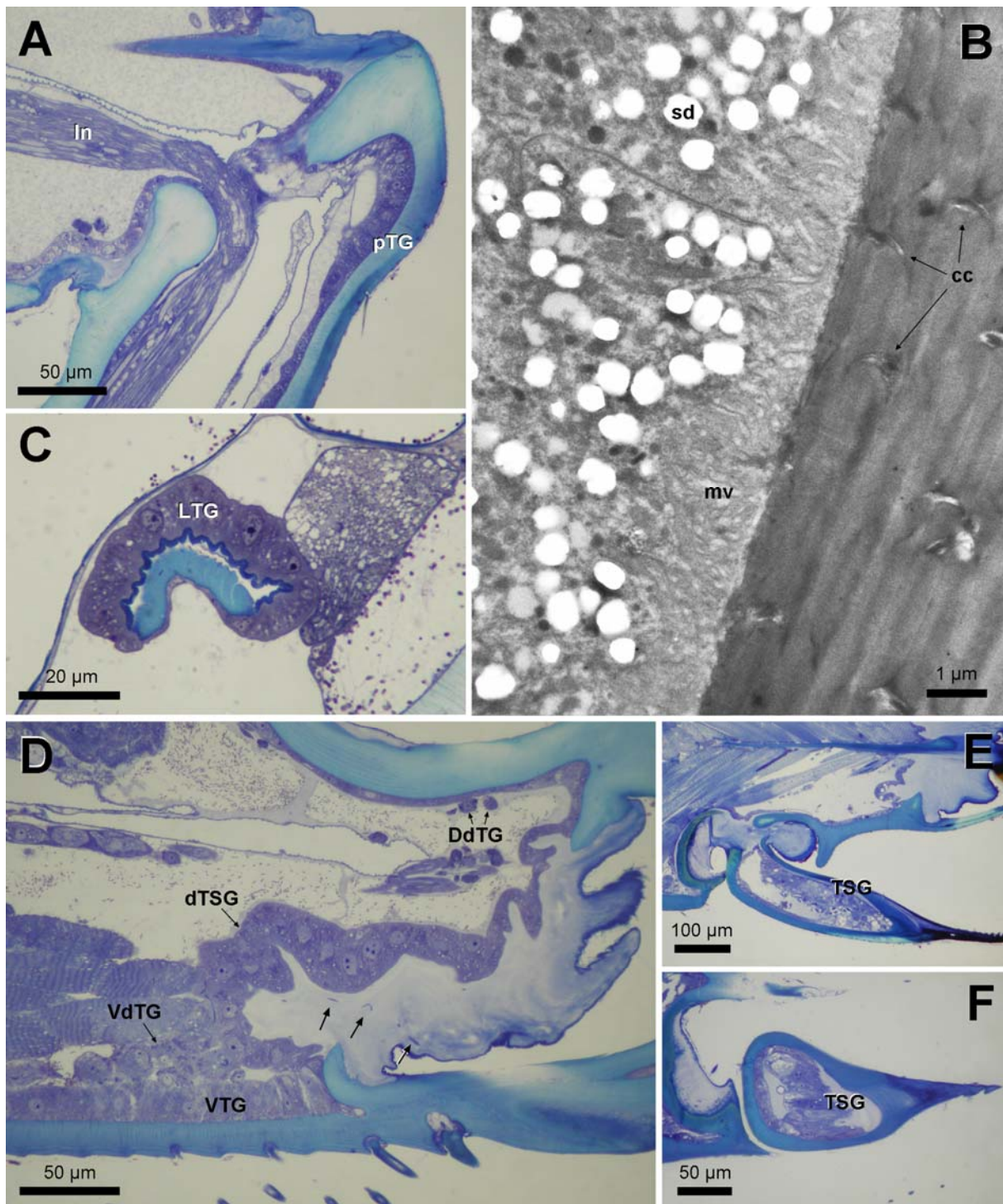


Fig. 4. Tibial glands. **A.** Junction between femur and tibia in worker hindleg, showing proximal tibial gland (PTG). **B.** Ultrastructure of proximal tibia gland in midleg of virgin queen, showing cuticular cracks (cc) in cuticle. **C.** Cross section through tibial part of leg tendon gland (LTG) in midleg of virgin queen. **D.** Longitudinal section through distal part of tibia of male foreleg, showing dorsodistal tibial gland (DdTG), distal tibial sac gland (dTSG), ventrodistal tibial gland (VdTG) and ventral tibial gland (VTG). Arrows indicate ducts through intersegmental



membrane. **E.** Tibial spur in foreleg of mated queen with tibial spur gland (TSG).  
**F.** Tibial spur in worker foreleg with tibial spur gland (TSG). In: leg nerve, mv:  
microvilli, N: nucleus, sd: secretory droplets.

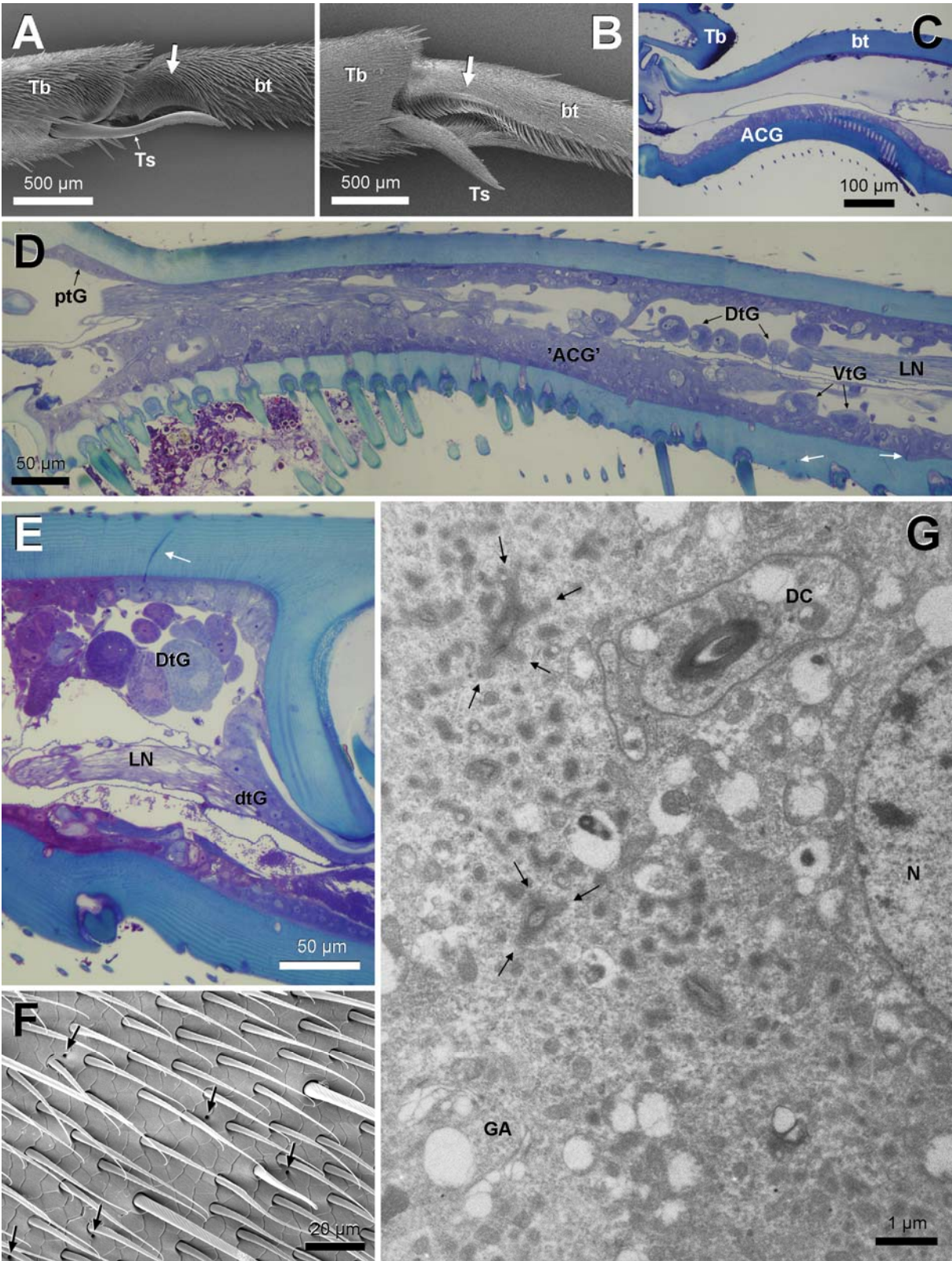


Fig. 5. Gland structures in the basitarsus. **A.** Foreleg antenna cleaner in a virgin queen at the junction between tibia (Tb) and basitarsus (bt) with a prominent tibial spur (Ts) and an opposite brush of stiff hairs (arrow). **B.** Similar structure in the hindleg of the same virgin queen. **C.** Longitudinal section through foreleg antenna cleaner of a mated queen with antenna cleaner gland (ACG). **D.** Similar section through proximal basitarsus of worker hindleg with equivalent of 'antenna cleaner gland'. Also note proximal, dorsal and ventral tarsomere glands (arrows show ducts of the latter). **E.** Longitudinal section through distal part of foreleg basitarsus in a virgin queen, arrow indicates duct of dorsal tarsomere gland. **F.** Scanning micrograph of upper surface of foreleg basitarsus of a worker, arrows show duct openings of dorsal tarsomere gland. **G.** Ultrastructure of dorsal tarsomere gland cell in foreleg basitarsus of a male, showing branched end apparatus (arrows) and Golgi apparatus.



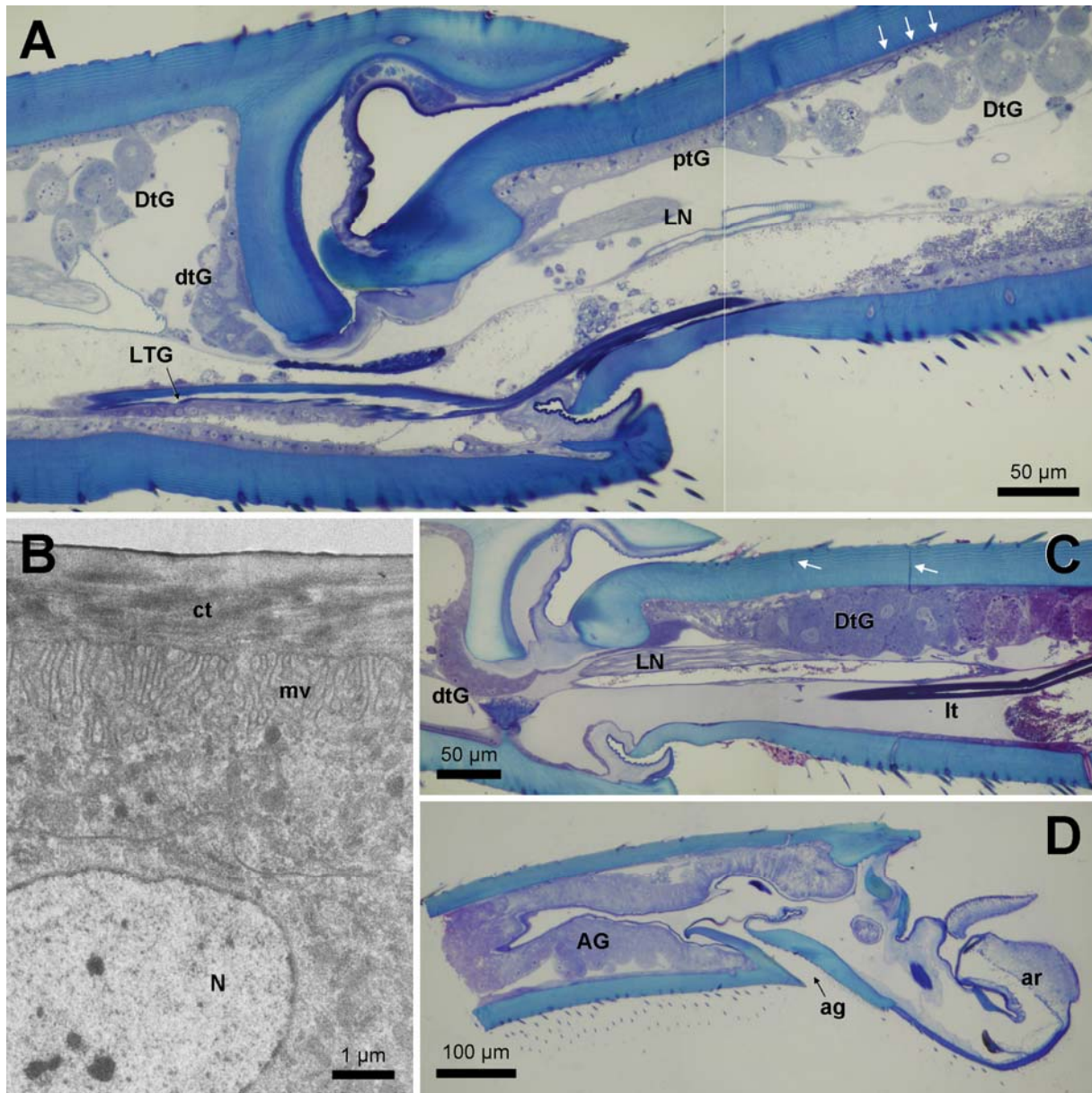


Fig. 6. Gland structures in the tarsomeres. **A.** Longitudinal section through t2-t3 junction in midleg of a mated queen; arrows indicate ducts of dorsal tarsomere gland. **B.** Ultrastructure of apical part of basitarsal tendon gland in foreleg of a male, showing microvillar border (mv) underneath cuticle (ct). **C.** Longitudinal section through proximal part of t3 in hindleg of a virgin queen with distal and dorsal tarsomere gland (arrows indicate ducts of the latter). **D.** Longitudinal section through pretarsus (= t5) of midleg of a virgin queen showing the arolium gland (AG). ACG: antenna cleaner gland. ag: arolium groove, ar: arolium, DC: duct cell, dtG: distal tarsomere gland, DtG: dorsal tarsomere gland, LN: leg nerve, It: leg tendon, LTG: leg tendon gland, N: nucleus, ptG: proximal tarsomere gland, VtG: ventral tarsomere gland.